the inhibition of serum dopamine β-hydroxylase by fusaric acid in vivo may become irreversible. Another possible explanation may be that fusaric aid inhibits the discharge of the enzyme from the sympathetic nerve endings. This problem remains for further investigation[9].

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Zusammenfassung. Die Dopamin-β-Hydroxylase-Aktivität im menschlichen Serum wurde nach der oralen Zufuhr von Fusarinsäure (50–300 mg) stark gehemmt.

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Non-Membrane-Bound Cytoplasmic Deposits in Krabbe Globoid Leukodystrophy: Further Evidence for a Revised Concept of Lysosomal Storage Diseases

In the past decade, biochemical studies have revealed that the essential defect underlying many of the inherited storage diseases is a genetically determined block in one or more degradative pathways, i.e., a lacking or extremely lowered activity of one or more catabolic enzymes. These are mainly hydrolases with acid pH optimum largely localized within cytoplasmic organelles defined by and comprised in the lysosome concept. It is, therefore, not astonishing that, in various thesaurismoses, the respective storage materials were found to be deposited for the most part inside membrane-bound cytoplasmic bodies or vacuoles of lysosomal nature. Accordingly, the term 'inborn lysosomal storage diseases' was introduced to designate metabolic errors of this kind[1,2]. As to the mechanisms by which the storage substances gain entrance into and can be concentrated within the lysosome system, two pathways have been postulated: cellular autophagy and endocytosis[1,2]. In the case of deficient activity of one or more acid hydrolases normally carried into autophagic and heterophagic vacuoles by primary lysosomes, the natural substrates of these enzymes cannot be cleaved during digestion of the vacuole contents, and thus remain as inert residues within the vacuoles. Repetition of such an incomplete degradative process must necessarily cause an accumulation of indigestible substances inside an increasing number of secondary lysosomes and/or residual bodies[1,2].

Krabbe globoid leukodystrophy (GLD) is in essence a hereditary disorder of galactocerebroside metabolism transmitted most probably as an autosomal recessive trait[3]. We examined electron microscopically a brain biopsy from a child with GLD, and we observed numerous cells containing the multiangular polymorphous inclusions which are said to be characteristic of Krabbe's disease and to consist for the most part of galactocerebrosides[4]. It was remarkable that these deposits were present by no means exclusively within membrane-bound lysosome-like bodies or vacuoles of variable size and appearance. Many inclusions were met with also lying free in the ground cytoplasm or embedded in non-membrane-bound patches of a fairly opaque substance (Figure). From the perusal of the pertinent literature it became obvious to us that this striking finding had already been observed by some other authors, who had studied the ultrastructural alterations in the central or peripheral nervous system of GLD patients prior to us[5–7]. In one paper it had even been emphatically stated that the cytoplasmic inclusions seen in a nerve biopsy from a child with GLD were never membrane-bound[8].

Till now, two different enzymes have been shown in human GLD to be consistently reduced in activity. The earlier detected one is lipid sulfotransferase, a biosynthetic enzyme which catalyzes the conjugation of galactocerebrosides with sulfate groups to form sulfatides. The other and later detected one is galactocerebroside β-galactosidase, a degradative enzyme which catalyzes the splitting of galactose from galactocerebrosides to form ceramides[9]. However, sulfotransferase deficiency was found to be largely restricted to the brain[3,9], whereas reduced activity of galactocerebroside β-galactosidase could be demonstrated in the CNS as well as in a variety of non-nervous tissues[3,10]. Moreover, galactocerebroside β-galactosidase could recently be shown to be partially deficient in cells and blood serum from heterozygous carriers of GLD[10]. All this together would suggest that the defect of this specific galactosidase is more likely to be the primary, genetically determined cause of Krabbe's disease. Nevertheless, lowered lipid sulfotransferase activity in the brain must be regarded also as an integral feature of human GLD[9].

The fact that cytoplasmic galactocerebroside deposits may occur, at least in some cases and/or in certain stages of human GLD, both inside and outside the lysosome system, seems, at first glance, to be incompatible with the general concept of inborn lysosomal storage diseases. In order to explain this morphological finding without

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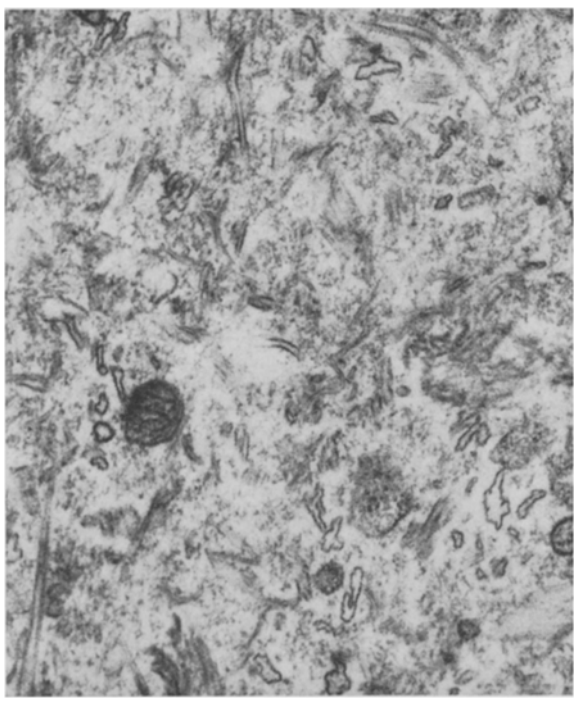
giving up completely the above concept, we should like to suggest the following. First of all, it should be emphasized that only one of the two enzymes involved in this pathological process, namely galactorecebroside β-galactosidase, is a lysosomal one. Lipid sulfotransterase, on the other hand, is not lysosome-bound but, like other transferases, it is closely associated with the smooth-surfaced endoplasmic reticulum[9]. One may suggest that, in the course of Krabbe's disease, deficient or absent activity of galactocerebroside β-galactosidase is responsible mainly for the accumulation of galactocerebrosides within membrane-bound vacuoles or bodies of lysosomal nature; lowered lipid sulfotransferase activity, in turn, accounts for the deposition of particulate galactocerebroside inclusions within the cytoplasmic matrix proper. Secondly, liberation of originally intralysosomal galactocerebroside deposits into the ground cytoplasm as a consequence of membrane labilization and lysosome leakage, must also be taken into consideration. Finally, it should be mentioned that, in Krabbe's disease, membrane-bound lysosomal structures may be only secondarily involved in the process of storage, as has been supposed in some other sphingolipidoses[11]. This would imply, of course, an initial extralysosomal stage of the deposited substances. Moreover, one would be compelled to assume that lysosomal hydrolases may be operative, at least to some extent and/or for short intervals, also out of the lysosome system in normal cell metabolism. Up to the present, no direct evidence exists for any of these hypotheses. However, we feel that one or more of them may be verified in the future. Be that as it may, the general concept of inborn lysosomal storage diseases evidently requires reconsideration and/or completion. In its present form, it does not satisfactorily explain all morphological findings thus far obtained in human GLD as well as in various other storage diseases.



Cytoplasmic portion of an inclusion-bearing cell in GLD brain biopsy material. Note polygonally shaped and needle-like profiles representing transversally and longitudinally sectioned deposits which obviously lack any membranous demarcation against the ground cytoplasm. ×30,000.

Zusammenfassung. Bei der Krabbeschen Leukodystrophie können die charakteristischen Zytoplasmaeinlagerungen sowohl innerhalb als auch ausserhalb membranbegrenzter lysosomaler Kompartimente vorkommen. Es wird versucht, für diesen Befund einige plausible Erklärungen zu geben. Ausserdem wird hervorgehoben, dass das allgemeine Konzept der angeborenen lysosomalen Speicherkrankheiten in seiner bisherigen Form nicht ausreicht, alle morphologischen Beobachtungen bei den verschiedenen Thesaurismosen befriedigend zu deuten.

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Fractionation and Characterization of Cell Nuclei from Rat Neocortex by Protein-DNA Ratios[1]

Several different isolation and fractionation methods have been developed for brain nuclei[2–4], but criteria for their differentiation are still controversial. The protein/DNA ratios found in the literature vary remarkably[4–6], and no information can be found on the problem of reproducibility. For our procedure we adopted a slightly modified method of Burdman[2], which gave reproducible results when a strict time table was observed. Since the morphological criteria described in the literature are clearly insufficient, we have characterized our nuclei by chemical parameters, and these will be compared with the findings of other authors.

Methods: (Times in parenthesis indicate min between the death of the animal and the next step of the procedure). 2 male rats (300–400 g) used for each experiment are anaesthesized and killed by decapitation. All further steps are carried out at 0–4°C. The combined neocortex (ca. 1.2 g) is homogenized (15 min) by hand (15 strokes) with 0.32 *M* sucrose containing 1 m*M* $MgCl_2$ and 1 m*M* KH_2PO_4, pH 6.5 (w/v 1:15), with a Teflon pestle in a Potter homogenizer. The homogenate is diluted to 50 ml with homogenization medium and centrifuged for 10 min at 900 g_{av}

[1] This investigation was supported by grant No. 89 of the Fritz Hoffmann-La Roche Foundation.
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